# The Adaptive Response in Radiobiology: Evolving Insights and Implications

## Sheldon Wolff

Department of Radiology, University of California San Francisco, San Francisco, California

The first of the regularly reproducible experiments to show that very low doses of ionizing radiation, like very low doses of chemical agents, could induce mechanisms whereby cells become better fit to cope with subsequent exposures to high doses were carried out on the induction of chromosome aberrations in cultures of human lymphocytes. If cells that had been exposed to a very low dose (1 cGy ) of X rays were subsequently exposed to a relatively high dose (1 Gy), approximately half as many chromosome breaks were induced. Subsequent experiments showed that this adaptive response to low doses requires a certain minimal dose before it becomes active; occurs only within a relatively small window of dose; is dose-rate dependent; and depends on the genetic constitution of the people or animals exposed, with some being unresponsive. It was further shown that the response to the low-dose preexposure was not instantaneous but took approximately 4 to 6 hr to become fully active, and could be prevented if during this period protein synthesis was inhibited, i.e., a necessary protein (enzyme) was being induced. In fact, subsequent experiments with two-dimensional gel electrophoresis showed new proteins in cells irradiated with 1 to 2 cGy. The adaptation induced by low doses of radiation was therefore attributed to the induction of a novel efficient chromosome break repair mechanism that if active at the time of challenge with high doses would lead to less residual damage. This hypothesis was strengthened by a series of experiments in which it was found that inhibitors of poly(ADP-ribose)polymerase, an enzyme implicated in DNA strand break rejoining, could prevent the adaptive response. Although the phenomenon is well established in cellular systems, it is still problematical as to whether or not it will have any utility in establishing risks of ionizing radiation to humans. Newer experiments have now been carried out on the mechanisms underlying the effect and whether or not the effect can manifest itself as a decrease in the number of induced cancers and radiation-induced mortality. Experiments with restriction enzymes now indicate that double-strand breaks in DNA can be triggering events in adaptation. In addition, preliminary experiments on the survival of whole-body irradiated mice have shown that multiple exposures to low adapting doses can have profound effects on survival, and other experiments have shown that adaptation can affect the induction of thymic lymphoma in irradiated mice. It therefore appears that the initial experiments behind the adaptive response have led to a vigorous worldwide effort to understand the basic mechanisms behind it. This effort is stimulated both by a desire to understand the basic cell biology behind the response and a desire to see if indeed this phenomenon affects the estimation of risks of low-level radiation exposure. — *Environ Health Perspect* 106(Suppl 1): 277–283 (1998). *http://ehpnet1.niehs.nih.gov/1998/Suppl-1/277-283wolff/abstract.html*

Key words: adaptive response, chromosome aberrations, cytogenetics, adaptation, ionizing radiation, human lymphocytes, restriction enzymes, repair, radiation risks

## Introduction

Because of the uncertainties surrounding the shapes of dose–response curves at low levels of ionizing radiation, the usual model for establishing risks of radiation at very low doses where it is not possible to obtain statistically valid experimental data has been to extrapolate the results obtained at high doses to those expected in the low-dose region. This procedure has been controversial as to whether the extrapolation

*a*) should be linear, because the physical dose by definition increases linearly, *b*) should involve a threshold, which could result from biological processes that modify the initial responses or perhaps even reflect the nature of the biological event (e.g., multistep carcinogenesis), or *c*) should be greater than linear, because radiation-induced homeostatic processes could be inactive at very low doses. Although there are underlying uncertainties in all these procedures, the linear hypothesis has been the one most accepted by regulatory agencies as being a prudent way to estimate risk and thus prevent undue harm to the public.

As early as 1976 some high-dose experiments with algae and desmids indicated that a complex biology could possibly modify a simple linear response to ionizing radiation. Experiments of this type, which invoked the induction of the repair of potentially lethal damage, however, did not speak to what might possibly happen at low doses where data were unobtainable and for which the above-mentioned uncertainties existed.

The first of the regularly reproducible experiments to show that very low doses of ionizing radiation, like very low doses of chemical agents, could induce mechanisms whereby cells became somewhat refractory to the induction of damage by subsequent exposures to high doses were carried out on the induction of chromosome aberrations in cultures of human lymphocytes.

As a result of this work, others carried out similar experiments with other biological end points and other types of cells, and the evidence kept mounting that this was a newly discovered phenomenon of great intrinsic interest and possibly of practical importance in understanding what might actually occur at very low doses and how this might affect risk estimation. Soon, enough evidence on this adaptive response to low doses had been gathered from all over the world for the United Nations Committee on the Effects of Atomic Radiation (UNSCEAR) to point out that there was no doubt about the phenomenon's occurrence at the cellular level, but that it was still problematic if it had practical consequences in estimating damage to populations and, in particular, in estimating cancer risks (*1*).

## Early Experiments

In the initial experiments on the induction of adaptation by low doses of ionizing radiation, human lymphocytes that had

incorporated tritiated thymidine (2) were exposed to low doses of chronic radiation as the tritium disintegrated, and the cells were subsequently exposed to the relatively high dose of 150 cGy of X rays. Approximately half as many chromosome aberrations were induced as in cells that had not incorporated the radioisotope. This result with very low doses of radiation was reminiscent of the adaptive response found in cells treated with low chronic doses of alkylating agents, which made those cells less sensitive to high doses of the same or other alkylating agents (3,4). Subsequent experiments in which the amount of radioisotope was greatly reduced soon showed that the adapting, or conditioning, dose need not be chronic because a single instantaneous disintegration of incorporated tritium could bring it about (5). Further experiments then showed that preexposure of the lymphocytes to X-ray doses as low as 1 cGy could cause the cells to adapt (6).

In experiments with chemical agents it was also found that cross-adaptation occurred. That is, exposure of cells to low doses of radiomimetic chemicals, alkylating agents, cross-linking agents, or ionizing radiations all could lead to a decrease in the cell's sensitivity to the same agent or any of the others (7).

The adaptation induced by low doses of radiation was attributed to the induction of a novel, efficient chromosome-break repair mechanism that, if present at the time of the challenge with high doses of radiation, would result in less damage after the exposure (2,5). This hypothesis was strengthened by experiments in which it was found that an inhibitor (3-aminobenzamide) of poly(ADP-ribose) polymerase, an enzyme implicated in DNA strand break rejoining, prevented the adaptive response (5,6). It did this even when it was administered after the challenge dose but within the time before induced chromosome breaks rejoined, which indicated that the decrease was caused not by a change in the initial sensitivity of the cells but by a postexposure phenomenon such as repair (5).

It was soon found that the response to a preexposure to low doses of radiation was not instantaneous but took some 4 to 6 hr to become fully active (8), and that this response could be prevented during this period if protein synthesis was inhibited (9). That is, a necessary protein (enzyme) was being induced, and subsequent experiments with two-dimensional gel electrophoresis indeed, did show new proteins in cells irradiated with 1 to 2 cGy

(10) and one-dimensional gel electrophoresis indicated that a protein that binds specifically to radiation-damaged DNA is produced (11).

## New Work

In spite of all these advances attempts are still being made to see if explanations other than induced repair can account for the phenomenon, and to define at the molecular level the exact mechanism(s) behind adaptation. In addition attempts are also being made to see if the adaptive response can affect the survival of animals after whole-body irradiation and if fewer cancers are induced in preexposed animals. During the last several years my laboratory has carried out several experiments designed to obtain further information on various aspects of the adaptive response.

### Differential Display of mRNAs

For example, further attempts have been made to find a method that could lead to identification of the molecular processes involved in the repair manifested as adaptation. Because Boothman and his colleagues have found changes in gene transcription levels after exposure to ionizing radiations, (12,13), experiments now have been carried out to determine if the low doses of X rays that result in adaptation can indeed induce changes in the transcription level of genes involved in regulation of the DNA repair postulated to be involved in adaptation. In these experiments, human lymphocytes exposed to an adapting dose of 2 cGy of X rays were analyzed to see if different species of mRNAs were present in the irradiated cells, as would be true if gene activity were induced. A suitable set of primers is used. One of these is an oligo dT that anchors to the polyA tails of the mRNAs; the other is one of a group of arbitrary decamers that anneals at various positions relative to the attached oligo dT primer. In this technique, when four anchored dT primers are used in conjunction with at least 25 upstream decamers, almost all of the expressed mRNA species can be reverse transcribed to cDNA (14,15). These cDNAs are then amplified by the polymerase chain reaction with arbitrary decamer primers, and the DNA fragments representing the 3′ termini of mRNAs are separated by size on a denaturing polyacrylamide gel. Any cDNA fragments corresponding to differentially expressed genes that produced mRNA in irradiated cells were compared with the cDNA fragments found in unirradiated
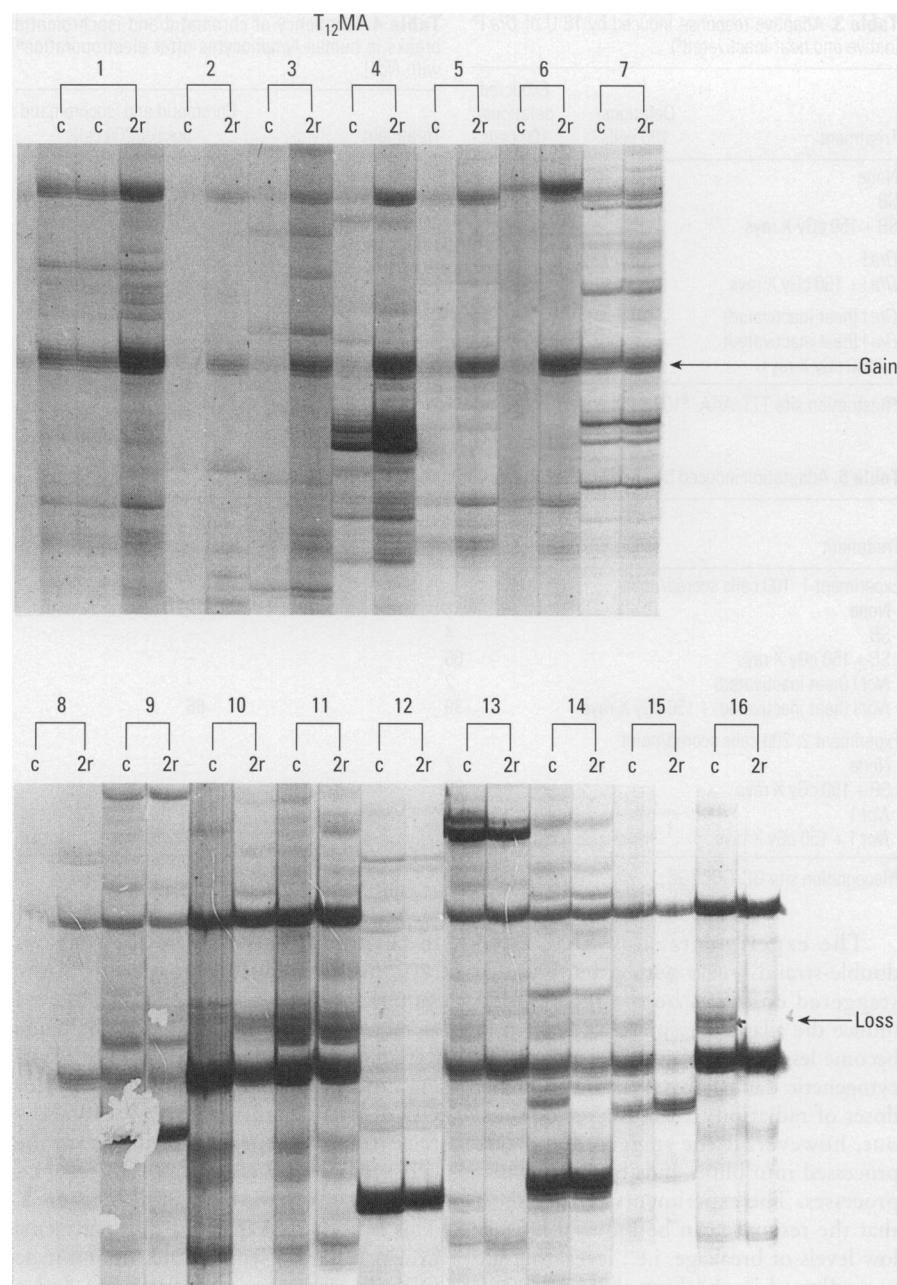
(control) cells. It has been found (Figure 1) that the irradiated cells have some mRNAs not present in control cells. The corresponding cDNAs of these represent appropriate candidate genes that could be involved in the repair manifested as adaptation. The irradiated cells also lack some mRNAs present in the control cells. In addition some cDNAs made from mRNAs were found in control cells but not in adapted cells. These, then, could represent candidate suppressor genes that could be involved in the regulation of the (DNA) repair. These experiments are providing access to the DNA involved in the adaptive response and indicate that the response might be attributable either to the induction of new enzymes or to the loss of a repressor in the irradiated cells.

### Induction of the Adaptive Response by DNA Double-strand Breaks

All the chemicals and radiations that induce the adaptive response are not specific in that they produce a spectrum of lesions in DNA such as base damage, single-strand breaks, and double-strand breaks that in principle could be responsible for the phenomenon. Because ionizing radiations are efficient inducers of DNA double-strand breaks and the adaptive response is mediated by poly(ADP-ribose) polymerase, an enzyme stimulated in response to such breaks, it is thought that double-strand breaks alone might be the lesions responsible. To see if they alone can induce adaptation, we carried out experiments with restriction enzymes, which unlike ionizing radiations and radiomimetic chemicals, only induce one specific type of lesion, DNA double-strand breaks, by binding to specific DNA sequences, or recognition sites, and cleaving the DNA at these sites.

The experiments were carried out with human lymphocytes in which restriction enzymes were introduced into the cells by electroporation to produce different numbers of DNA double-strand breaks of various types. Cells into which only the storage buffer for the enzymes was electroporated served as the controls. Alu I, which induces a large number of blunt-end DNA double-strand breaks at the recognition site AG/CT; Dra I, which induces fewer double-strand breaks at the recognition site TTT/AAA; and Not I, which induces only very few staggered-end double-strand breaks at the recognition site GC/GGCGC, were used.

All three of the restriction enzymes induced adaptation in that they reduced

**Figure 1.** Polyacrylamide gel of cDNAs obtained by the differential display of mRNA technique. $T_{12}MA$ was the oligo dT primer that bound to the poly dT tails of the mRNA. Columns 1 to 16 were obtained with 16 different arbitrary upstream decamer primers. Abbreviations: c, control cells; 2r, irradiated cells.

**Table 1.** Adaptive response induced by 5 U of Alu I[a] (native and heat inactivated[b]).

| Treatment | Deletions/ 100 cells | Expected deletions[c]/ 100 cells |
|---|---|---|
| SB | 2 | – |
| SB + 150 cGy X rays | 69 | – |
| Alu I | 35 | – |
| Alu I + 150 cGy X rays | 60 | 102 |
| Alu I (heat inactivated) | 12 | – |
| Alu I (heat inactivated) + 150 cGy X rays | 59 | 79 |

SB, storage buffer. [a]Recognition site AG/CT. [b]100 cells/point. [c]Sum of deletions seen in slides of cells exposed to 150 cGy after electroporation in presence of SB, plus those seen in slides electroporated with Alu I alone. Because both of these numbers include the control (SB) level of deletions, one control level is subtracted.

DNA fragments on an agarose gel. DNA digested with active Alu I showed multiple bands, whereas DNA exposed to the heat-inactivated enzyme did not, i.e., was uncut (Figure 2).

That the heat-inactivated enzyme, which induced adaptation, might have renatured inside the cell and thus regained its ability to cleave DNA was shown in experiments on the induction of mutations in the shuttle vector pHAZE, which can replicate in both mammalian and bacterial cells. It was exposed to heat-inactivated enzyme while it was maintained as an episome in human lymphoblastoid cells (Raji) cells. The plasmid recovered from the host contained enzyme-induced mutations selected in *Escherichia coli* (Table 2). Therefore, heat-inactivated Alu I, which had no enzymatic activity *in vitro* (Figure 2), did induce mutations in pHAZE after its introduction into cells by electroporation. The experiments indicate that once inside the cell, the denatured enzyme can renature and become able to cut DNA. They further indicate the importance of using an unrelated nonenzymatic protein such as bovine serum albumin as a proper control to ascertain whether the effects observed after exposure to a restriction enzyme can be attributed to a nonspecific effect of proteins in general.

Similar experiments with Dra I, which induces far fewer DNA strand breaks and thus fewer chromosome aberrations than Alu I, reduced by approximately 45% the number of chromatid breaks subsequently induced by X rays (Table 3), indicating once again that blunt-end double-strand breaks themselves are lesions capable of inducing the adaptive response. As before,

the number of chromosome breaks produced by a subsequent exposure to 150 cGy of X rays. When the cells were pretreated with Alu I, the yield of chromatid aberrations found after 150 cGy of X rays was 60%, whereas the sum of the aberrations induced by Alu I alone and 150 cGy of X rays alone was 102% (Table 1).

In these experiments the controls always consisted of cells electroporated with the restriction enzymes' storage buffers, which contain bovine serum albumin. Attempts to introduce a more specific control in which heat-inactivated Alu I was introduced into the cells showed that the heat-treated enzyme, too, led to adaptation (Table 1). That the heat-inactivated enzyme was inactive in cleaving DNA was confirmed by assaying for restriction enzyme activity by incubating DNA from the plasmid pHAZE with active or heat-inactivated Alu I *in vitro* and resolving the

**Figure 2.** Cleavage of DNA by native but not heat-inactivated *Alu* I. Lane 1, uncut pHAZE; lane 2, *Alu* I, lane 3, heat-inactivated *Alu* I, lane 4, 1-kb ladder.

**Table 2.** Frequency of *lac-Z* mutants induced in intra-cellular pHAZE by previously heat-inactivated *Alu* I.[a]

| Treatment | Mutation frequency, $\times 10^{-4}$ |
| --- | --- |
| SB | 2.74 |
| *Alu* I, 200 U | 5.89 |
| Heat-inactivated *Alu* I, 500 U | 7.28 |

[a]Recognition site AG/CT.

**Table 3.** Adaptive response induced by 10 U of *Dra* I[a] (native and heat-inactivated[b]).

| Treatment | Deletions/ 100 cells | Expected deletions/ 100 cells |
| --- | --- | --- |
| None | 3 | – |
| SB | 2 | – |
| SB + 150 cGy X rays | 54 | – |
| *Dra* I | 8 | – |
| *Dra* I + 150 cGy X rays | 41 | 60 |
| *Dra* I (heat inactivated) | 4 | – |
| *Dra* I (heat inactivated) + 150 cGy X rays | 37 | 56 |

[a]Restriction site TTT/AAA. [b]100 cells/point.

**Table 4.** Frequency of chromatid and isochromatid breaks in human lymphocytes after electroporation[a] with *Not* I.

| Treatment | Chromatid and isochromatid breaks/200 cells |
| --- | --- |
| Control | 2 |
| SB | 17 |
| *Not* I, U | |
| 200 | 1 |
| 100 | 9 |
| 25 | 11 |
| 12.5 | 8 |

[a]Electroporation was carried out at 48 hr of culture (i.e., after stimulation with phytohemagglutinin [PHA]). All cultures were harvested at 72 hr.

**Table 5.** Adaptation induced by 12.5 U of *Not* I[a] (native and heat inactivated).

| Treatment | Deletions, no | Expected deletions, no |
| --- | --- | --- |
| Experiment 1: 100 cells scored/point | | |
| None | 3 | – |
| SB | 4 | – |
| SB + 150 cGy X rays | 66 | – |
| *Not* I (heat inactivated) | 2 | – |
| *Not* I (heat inactivated) + 150 cGy X rays | 39 | 66 |
| Experiment 2: 200 cells scored/point | | |
| None | 2 | – |
| SB + 150 cGy X rays | 79 | – |
| *Not* I | 8 | – |
| *Not* I + 150 cGy X rays | 51 | 85 |

[a]Recognition site GC/GGCCGC.

the previously heat-inactivated enzyme could cause the effect.

Various concentrations of the restriction enzyme *Not* I (12.5 to 200 U/0.8 ml) were introduced into the cells by electroporation 48 hr after stimulation with phytohemagglutinin, and the cells were scored 6 hr later to see if chromatid aberrations could be induced. This restriction enzyme, which cuts DNA infrequently, did not induce a significant number of chromosome aberrations even at the highest dose (Table 4). It did, however, induce the adaptive response in cells subsequently exposed to 150 cGy (Table 5) even at the lowest concentration, which reduced the number of X-ray-induced aberrations by 40%.

The experiments show that DNA double-strand breaks with either blunt or staggered ends can be the lesions that induce the adaptive response whereby cells become less susceptible to the induction of cytogenetic damage by exposure to higher doses of radiation. It cannot yet be ruled out, however, if the staggered ends are processed into blunt ends by intracellular processes. The experiments further show that the response can be induced by very low levels of breakage, i.e., levels that are not reflected in an observable increase in chromosome aberrations.

## Independence of the Adaptive Response on Induced Cell-cycle Delays that Allow More Time for Repair

End points other than chromatid breaks have been used in experiments on the adaptive response. For instance, experiments on cellular survival responses have been carried out (12,16). It has been proposed that the adapting dose, even though very low, induces an effect on cell cycling, perhaps by signal transduction mechanisms, and that this is reflected in changes in sensitivity to the killing effects of radiation. For such

mechanisms to account for the adaptive response that causes a reduction in the number of chromatid aberrations observed at metaphase only 6 hr after a challenge dose of 150 cGy, however, a 2-cGy adapting dose would have to induce a $G_2$ (not $G_1$) delay that purportedly would allow more time for repair to occur before the cells challenged with 150 cGy reached metaphase where they could be scored. This hypothesis was tested in experiments in which human lymphocytes from a male (XY chromosome constitution) and a female (XX chromosome constitution) were mixed in various combinations after being exposed to either no radiation, an adapting dose, a challenge dose, or both an adapting and a challenge dose. The mixtures were made at the time of the challenge dose and the cells were cocultured for 6 hr until fixation, at which time the proportions of cells at metaphase from the male and the female could be observed cytologically. The lymphocytes from both sexes showed typical adaptive responses when preexposed to 2 cGy of X rays before being challenged with 150 cGy (Table 6).

Regardless of the treatment, the percentage of cocultivated male and female

cells reaching metaphase stayed the same (Table 7). That is, when 200 consecutive metaphases were observed 6 hr after mixing, 55 untreated male cells and 145 untreated female cells were found. When both groups of cells received 150 cGy of X rays before mixing, 48 male cells and 152 female cells appeared at metaphase, which is not significantly different from the 55 and 145 cells seen with no radiation exposure. Exposing cells to an adapting dose before the 150 cGy challenge did not in any combination affect the speed at which the cells appeared at metaphase, so that the proportion of male cells and female cells did not change.

These experiments indicate that adaptation is not caused by a change in the rate of cell progression to mitosis after a challenge dose, and are a further indication that cell stage sensitivity is not a factor in the adaptive response observed as decreased cytogenetic damage.

## Worldwide Experiments with Other End Points

Stimulated by the early work, other groups soon found that this adaptation phenomenon

**Table 6.** Adaptive response in human lymphocytes from a male and a female.

| Treatment | Deletions/cells, male | Deletions/cells, female |
|---|---|---|
| Control (unirradiated) | 2/100 | 1/100 |
| 2 cGy | 3/100 | 5/100 |
| 150 cGy | 39/100 | 42/100 |
| 2 cGy + 150 cGy | 27/100 | 27/100 |

The adapting dose (2 cGy) was administered at 24 hr of culture (i.e., after stimulation with PHA), the challenge dose (150 cGy) at 48 hr. Cells were fixed at 54 hr.

**Table 7.** Lack of effect of adapting dose of 2 cGy of X rays on cell progression of cells challenged with 150 cGy of X rays.

| X-ray dose, cGy | | Cells at metaphase | |
|---|---|---|---|
| Male | Female | Male | Female |
| 0 | 0 | 55 | 145 |
| 2 | 0 | 58 | 142 |
| 2 | 2 | 53 | 147 |
| 2 | 2 | 54 | 146 |
| 150 | 150 | 48 | 152 |
| 2 + 150 | 150 | 52 | 148 |
| 150 | 2 + 150 | 54 | 146 |
| 2 + 150 | 2 + 150 | 53 | 147 |

The adapting dose (2 cGy) was administered at 24 hr of culture (i.e., after stimulation with PHA), the challenge dose (150 cGy) at 48 hr. Equal volumes of each culture then were mixed and the cells cocultured for another 6 hr. Colcemid (2 x 10⁻⁷ M) was present for the last 2 hr. Two hundred metaphase cells were scored to determine whether they came from the male (XY) or from the female (XX).

was not restricted to the induction of chromatid deletions. For instance, experiments with the *HPRT* locus in human lymphocytes showed that exposure to tritiated thymidine (*17*) or 1 cGy of X rays (*18*) could markedly decrease the number of mutations induced by subsequent high doses of radiation. The response to low X-ray doses virtually eliminated the effects of the challenge dose.

Because radiation-induced mutations can be either small point mutations or larger chromosomal events such as deletions (which predominate), experiments were now carried out to determine the nature of the mutagenic events subject to adaptation. In the normal human lymphoblastoid cell line AHH-1, Rigaud et al. (*19*) examined 94 γ-ray-induced mutants by Southern blot analysis after digestion of the DNA by the restriction enzymes *Pst* I or *Eco* RI. They found a 4-fold reduction in the number of mutants induced by 4 Gy in cells pretreated with 2 cGy of γ-rays (Table 8). Molecular analysis of the nature of the mutations showed that 78% of the mutants induced by 4 Gy alone had detectable changes in the gene, i.e., had deleted bands or new bands after electrophoresis. In cells preexposed to 2 cGy of γ-rays, however, the proportion of mutants induced by 4 Gy that were characterized by loss or rearrangement of the gene was reduced to 42% (Table 9). As expected, the proportion of mutants that had unchanged restriction fragment patterns increased

**Table 8.** Adaptive response of human lymphoblastoid cells (AHH-1) induced by a preexposure to 2 cGy of γ-rays: *HPRT⁻* mutations.

| Treatment | Mutant frequency | |
|---|---|---|
| | Observed, ×10⁻⁶ | Induced, ×10⁶ |
| Control | 11.5±2.7 | – |
| 0.02 Gy | 8.9±2.8 | – |
| 4.0 Gy | 70.7±21.4 | 61.8 |
| 0.02 Gy+4.0 Gy | 24.4±8.6 | 15.5 |

Exponentially growing cells were exposed to γ-rays from ¹³⁷Cs. The interval between the low adapting dose and the high challenge dose was 6 hr. Data from Rigaud et al. (*19*).

accordingly. Thus, when the cells were preexposed to a low adapting dose, a change in the molecular spectrum of radiation-induced mutations was found. The preferential decrease in those premutational lesions that led to deletions is consistent with the interpretation of the early work that very low doses of radiation induce a chromosome break/repair mechanism.

Consistent with this is the work carried out by Zhou and colleagues (*20*) on the induction of *HPRT* mutations as well as the repair of DNA double-strand breaks in DNA in a mouse mammary carcinoma cell line (SR-1). When cells were irradiated with 0.01 Gy of γ-rays and then challenged with 3 Gy 18 or 24 hr later, approximately half as many mutations were induced as when cells were irradiated with only 3 Gy of γ-rays. Furthermore, the rate of repair of DNA double-strand breaks—the lesions responsible for chromosomal breaks (*21–23*)—increased in cells that had been preexposed.

The data on induced mutations illustrate two other known aspects of adaptation. That is, after the adapting dose it takes time for the induction to occur and once induced it disappears with time. The SR-1 data show that in this system the effect takes more than 6 hr to become operable; it then disappears if 48 hr elapse between the two doses, i.e., the effect lasts between 30 and 41 hr. As noted earlier, previous experiments on the induction of chromatid deletions in human lymphocytes showed that in those cells it took 4 to 6 hr for adaptation to become fully operable (*8*) and the effect was found to last for three cell cycles, about 40 hr.

In other mutational studies, Fritz-Niggli and Schaeppi-Buechi (*24*) showed that dominant lethal mutations induced *in vivo* in the classical genetic organism *Drosophila melanogaster* were also subject to the adaptive response. Repair-proficient (yw) as well as repair-deficient (mei 41, mus 302) strains showed the effect. That is, not only does adaptation occur *in vivo* and in germ cells, but as earlier noted it is unrelated to

**Table 9.** Distribution of types of *HPRT⁻* mutants after γ-rays.

| Treatment | Mutants, no | | | | |
|---|---|---|---|---|---|
| | Analyzed | Unchanged | Deletions | Rearranged: partial deletion ± new band | Total changed |
| Control | 24 | 17 (71%) | 1 (4%) | 6 (25%) | 7 (29%) |
| 0.02 Gy | 17 | 10 (59%) | 2 (12%) | 5 (29%) | 7 (41%) |
| 4.0 Gy | 27 | 6 (22%) | 7 (26%) | 14 (52%) | 21 (78%) |
| 0.02 Gy + 4.0 Gy | 26 | 15 (58%) | 6 (23%) | 5 (19%) | 11 (42%) |

Data from Rigaud et al. (*19*).

the well-known standard postreplication and excision repair mechanisms.

Other end points that are the result of chromosomal damage have also been observed. Ikushima found a reduction in the number of micronuclei induced in V79 Chinese hamster cells by radiation if the challenge dose of 1 cGy was preceded by an exposure to either tritiated thymidine (25) or 5 cGy of γ-rays (26). Azzam et al. (27) also found this same end point to be reduced in a normal human fibroblast line (AG1522) exposed to chronic radiation (4.25 Gy at 0.003 Gy/min) before being challenged immediately with 4.25 Gy given in less than 2 min.

As might be expected from the results obtained in *Drosophila*, adaptation does not appear to be restricted to cultured cells; it also can occur when animals are irradiated *in vivo*. Thus, Cai and Liu (28) first showed that adult male Kunming mice preirradiated with 1 cGy of X rays had fewer chromatid deletions induced in their somatic cells (bone marrow) and germ cells (spermatocytes) by a challenge dose of 75 cGy given 2.5 to 3 hr later. When female C57Bl6 mice were used, adapting doses as low as 2 cGy were effective, although in this experiment no germ cells were scored.

Experiments with yet another strain of mice (white SHK) by Gaziev and co-workers (29) showed that chronic irradiation, as used in Azzam et al.'s experiments with human fibroblasts (27), could also be effective in whole animals; i.e., the number of micronuclei induced in the bone marrow by 1 Gy of γ-rays was reduced when the mice were chronically irradiated with doses ranging from 12 to 500 cGy.

In general, however, not all individuals or strains of animals are equally responsive to adapting doses. In humans, individual variability was found by Sankaranarayanan et al. (30), who observed the adaptive response in 8 of 9 subjects tested, and by Bosi and Olivieri (31), who observed the response in 14 of 18 subjects tested. Similarly, strain differences have been found in experiments with mice. Wojcik et al. (32) observed a decrease in the aberrations found in lymphocytes of only one-third to one-half of the C57Bl6 mice that had been preexposed *in vitro* to 10 cGy of γ-rays at 32 hr of culture and were then challenged with 1.5 Gy at 48 hr. No other combinations of doses or times were tested. When, however, they carried out similar experiments with various protocols in the Heiligenberger inbred strain of mice, no such effect was found. When these results

are compared with those of Cai and Liu (28), Gaziev et al. (29), and Zhou et al. (20), it appears likely that the genetic constitution of the mouse strains can be a determining factor in their responsiveness.

The apparent genetic variability that determines whether or not a person or a strain of animal will react to a low dose of radiation is consistent with the hypothesis that genetically competent cells have a damage-inducible repair mechanism that can affect how the cell responds to a subsequent insult. Experiments have shown that in human lymphocytes (10), V79 cells (26), and human U1-Mel cells (12), new proteins are induced after exposure to low doses of radiation, presumably by activation of genes.

The mechanisms proposed for the adaptive response have also been invoked to explain the fine structure now noted in dose–effect curves for survival of irradiated mammalian cells in culture (33). When careful plating techniques are used to define the low-dose region of the survival curves, the cells exhibit extreme sensitivity to low doses that is not predicted by extrapolating the response from higher doses backward. As the radiation doses increase beyond about 0.3 Gy, radioresistance appears; this becomes maximal at doses of about 1 Gy. It is thought that it is this induced radioresistance that characterizes the usual ordinary cell survival curves. Experimental treatments that inhibit the adaptive response also inhibit the induction of this radioresistance, strengthening the relation to adaptation.

Cytogeneticists long have known that chromosome aberrations constitute an end point that could be related to the induction of cancer, although there was no rationale to connect them causally. The discovery of oncogenes and the exquisite control mechanisms by which translocations of oncogenes to promoter regions on other chromosomes could lead to activation, as in the case of Burkitt's lymphoma, or by which deletions of a suppressor locus could also lead to activation, as in the case of retinoblastoma, gave a direct mechanism through which chromosomal or cytogenetic damage could lead to cancer. Therefore, it is not unreasonable to think that an adaptive response that reduces cytogenetic damage might also reduce cancer. Because of the long latent period for cancer induction, however, it has not yet been determined whether adaptation does, indeed, lower cancer rates in human populations preexposed to low doses, and as the UNSCEAR report noted,

it is not clear whether or not the adaptive response will have any utility for the development of radioprotection guidelines. As a result a worldwide effort is being carried out to see if low-dose preexposures can modify the amounts of cancer induced in irradiated animals or even increase the survival of those lethally irradiated. Preliminary experiments now indicate that both possibilities might occur.

In India, Bhattarcharjee (34) found that when he preirradiated Swiss mice for 5 days with γ-rays at the rate of 1 cGy/day, thymic lymphoma was induced in 16% (8/50) of the animals. A high 2-Gy dose induced lymphomas in 46% (23/50) of the mice, whereas if the animals were preirradiated before exposure to the 2-Gy dose, only 16% of them developed the cancers; i.e., the preirradiation seemed to cancel the induction of thymic lymphoma by the high dose (Table 10).

In Japan, Yonezawa and co-workers (35) have carried out experiments in which they lethally irradiated 21-ICR mice with 8 Gy of X rays. About 30% survived 30 days after the irradiation. When the animals were preirradiated with 5 cGy of X rays, the survival rate increased to about 70%.

## Conclusion

Much remains to be learned about the adaptive response whereby exposure to very low doses of radiation results in less damage being induced by subsequent exposures to high radiation doses. Uncertainties still exist about many aspects of adaptation and its underlying mechanisms. What is certain, however, is that the phenomenon is real, and that a vigorous worldwide effort is now under way to understand the basic mechanisms involved. This effort is stimulated both by a desire to understand the basic cell biology behind the adaptive response and a desire to see if, indeed, this phenomenon affects the estimation of the risks of low-level radiation exposure.

**Table 10.** Adaptive response of $^{60}$Co γ-ray-induced thymic lymphoma in mice.

| Dose | Mice, no | Mice with thymic lymphoma, no |
|---|---|---|
| 0 | 50 | 0 |
| 5 × 1cGy | 50 | 8 (16%) |
| 2 Gy | 50 | 23 (46%) |
| (5 × 1cGy) + 2Gy | 50 | 8 (16%) |

Data from Bhattacharjee (34). Eight- to ten-week old Swiss mice were sacrificed 240 to 260 days after γ-ray exposure: 1 cGy/day for 5 days, 2 Gy 24 hr after last adapting dose.

## REFERENCES

1. United Nations Scientific Committee on the Effects of Atomic Radiation. 1994 Report to the General Assembly, With Annexes. Sources and Effects of Ionizing Radiation. New York:United Nations, 1994.
2. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223:594–597 (1984).
3. Samson L, Schwartz JL. Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines. Nature 287:861–863 (1980).
4. Samson L, Schwartz JL. The induction of resistance to alkylating damage in mammalian cells. In: Induced Mutagenesis: Molecular Mechanisms and Their Implications for Environmental Protection (Lawrence CW, ed). New York:Plenum Press, 1980;291–309.
5. Wiencke JK, Afzal V, Olivieri G, Wolff S. Evidence that the [$^3$H]thymidine-induced adaptive response of human lymphocytes to subsequent doses of X-rays involves the induction of a chromosomal repair mechanism. Mutagenesis 1:375–380 (1986).
6. Shadley JD, Wolff S. Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation. Mutagenesis 2:95–96 (1987).
7. Wolff S, Afzal V, Wiencke JK, Olivieri G, Michaeli A. Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation as well as to chemical mutagens that induce double-strand breaks in DNA. Int J Radiat Biol 53:39–47 (1988).
8. Shadley JD, Afzal V, Wolff S. Characterization of the adaptive response to ionizing radiation induced by low doses of X rays to human lymphocytes. Radiat Res 111:511–517 (1987).
9. Youngblom JH, Wiencke JK, Wolff S. Inhibition of the adaptive response of human lymphocytes to very low doses of ionizing radiation by the protein synthesis inhibitor cycloheximide. Mutat Res 227:257–261 (1989).
10. Wolff S, Wiencke JK, Afzal V, Youngblom J, Cortés F. The adaptive response of human lymphocytes to very low doses of ionizing radiation: a case of induced chromosomal repair with the induction of specific proteins. In: Low Dose Radiation: Biological Bases of Risk Assessment (Baverstock KF, Stather JW, eds). London:Taylor & Francis, 1989; 446–454.
11. Wolff S. Low-dose exposures and the induction of adaptation. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;21–28.
12. Meyers M, Schea RA, Petrowski AE, Seabury H, McLaughlin PW, Lee I, Lee SW, Boothman DA. Role of X-ray-inducible genes and proteins in adaptive survival responses. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;263–266.
13. Boothman DA, Meyers M, Fukunaga N, Lee SW. Isolation of X-ray-inducible transcripts from radioresistant human melanoma cells. Proc Natl Acad Sci USA 90:7200–7204 (1992).
14. Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967–971 (1992).
15. Liang P, Averboukh L, Pardee AB. Distribution and cloning of eukaryotic mRNAs by means of differential display: refinements and optimization. Nucleic Acids Res 21:3269–3275 (1993).
16. Meyers M, McCaffrey E, Odegaard J, Boothman D. X-ray-inducible transcripts and altered cell cycle regulation are involved in adaptive survival responses in human cells. Radiat Res 141:110–120 (1994).
17. Sanderson BJS, Morley AA. Exposure of human lymphocytes to ionizing radiation reduces mutagenesis by subsequent ionizing radiation. Mutat Res 164:347–351 (1986).
18. Kelsey KT, Memisoglu A, Frenkel D, Liber HL. Human lymphocytes exposed to low doses of X rays are less susceptible to radiation-induced mutagenesis. Mutat Res 263:197–201 (1991).
19. Rigaud O, Papadopoulo D, Moustacchi E. Decreased deletion mutation in radioadapted human lymphoblasts. Radiat Res 133:94–101 (1993).
20. Zhou PK, Liu XY, Sun WZ, Zhang YP, Wei K. Cultured mouse SR-1 cells exposed to low dose of γ-rays become less susceptible to the induction of mutagenesis by radiation as well as bleomycin. Mutagenesis 8:109–111 (1993).
21. Wolff S. The repair of X-ray-induced chromosome aberrations in stimulated and unstimulated human lymphocytes. Mutat Res 15:435–444 (1972).
22. Wolff S. Relation between DNA repair, chromosome aberrations, and sister chromatid exchanges. In: DNA Repair Mechanisms (Hanawalt PC, Friedberg EC, Fox CF, eds). New York:Academic Press, 1978;751–760.
23. Natarajan AT, Obe G, Van Zeeland AA, Palitti F, Meijers M, Verdegaal-Immerzeel EAM. Molecular mechanisms involved in the production of chromosomal aberrations. II: Utilization of Neurospora endonuclease for the study of aberration production by X-rays in $G_1$ and $G_2$ stages of the cell cycle. Mutat Res 69:293–305 (1980).
24. Fritz-Niggli H, Schaeppi-Buechi C. Adaptive response to dominant lethality of mature (class A) and immature (class B) oocytes of D. melanogaster to low doses of ionizing radiation: effects in repair-proficient (yw) and repair-deficient strains (mei 41$^{D5}$ and mus 302$^{D1}$). Int J Radiat Biol 59:175–184 (1991).
25. Ikushima T. Chromosomal responses to ionizing radiation reminiscent of an adaptive response in cultured Chinese hamster cells. Mutat Res 180:215–221 (1987).
26. Ikushima T. Radio-adaptive response: involvement of induction of specific gene expression by low doses of ionizing radiation. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;255–258.
27. Azzam EI, de Toledo SM, Raaphorst GP, Mitchel REJ. Radiation-induced radioresistance in a normal human skin fibroblast cell line. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;291–294.
28. Cai L, Liu S-Z. Induction of cytogenetic adaptive response of somatic and germ cells in vivo and in vitro by low-dose X-irradiation. Int J Radiat Biol 58:187–194 (1990).
29. Gaziev AI, Fomenko LA, Kozhanowskaja IK, Sirota NP. Radio-adaptive response in mice chronically exposed to low dose rate of γ-rays. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;259–262.
30. Sankaranarayanan K, v. Duyn A, Loos MJ, Natarajan AT. Adaptive response of human lymphocytes to low-level radiation from radioisotopes or X-rays. Mutat Res 211:7–12 (1989).
31. Bosi A, Olivieri G. Variability of the adaptive response to ionizing radiations in humans. Mutat Res 211:13–17 (1989).
32. Wojcik A, Bonk K, Müller W-U, Streffer C. Indications of strain specificity for the induction of adaptive response to ionizing radiation in mice. In: Low Dose Irradiation and Biological Defense Mechanisms (Sugahara T, Sagan LA, Aoyama T, eds). Amsterdam:Elsevier, 1992;311–314.
33. Joiner MC, Lambin P, Malaise EP, Robson T, Arrand JE, Skov KA, Marples B. Hypersensitivity to very-low single doses: its relationship to the adaptive response and induced radioresistance. Mutat Res 358:231–235 (1996).
34. Bhattarcharjee D. Role of radio-adaptation on radiation-induced thymic lymphoma in mice. Mutat Res 358:231–235 (1996).
35. Yonezawa M, Misonoh J, Hosokawa Y. Two types of X-ray-induced radioresistance in mice—presence of 4 dose ranges with distinct biological effects. Mutat Res 358:237–243 (1996).